gene, wherein the expression of said *bax* gene would sensitize said tumor cells.

REMARKS

Amendment

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

The 35 U.S.C. §112 Rejection

Claims 2-3 and 5-10 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The rejection is respectfully traversed.

The present invention is drawn to the induction of apoptosis and inhibition of cell growth by inducible expression of the Bax gene. Expression of Bax resulted in apoptotic cell death in human ovarian cancer cells but not in normal human peritoneal mesothelial cells (Examples 6-8, 30 and 31). Bax expression also sensitized refractory cancer cells to radiation (Examples 9, 20 and 21). It was further shown that overexpression of Bax significantly enhanced

chemotherapy-induced cytotoxicity in both established cell lines and primary tumor cells (Example 32). These data showed that overexpression of Bax alone or in combination with radiation or chemotherapy is useful in inhibition of tumor cell growth.

Applicants hereby submit data that shows the effects of Bax overexpression can be generalized to other types of tumor cells. As shown in the attached Declaration of the co-inventor, Dr. David Curiel, a synergistic radiosensitizing effect of Bax can be induced in refractory glioblastoma cell after gene delivery via recombinant adenovirus. This result was confirmed in an *in vivo* murine xenograft model of glioblastoma. Toxicity was selectively induced in tumors but normal astrocytes were spared, thereby demonstrating the clinical utility of combining *bax* gene delivery with radiotherapy for the treatment of malignant brain tumors.

Based on a fair reading of the data contained herein, Applicants submit that the methods claimed herein have reasonable correlation to the scope of the enablement provided. Accordingly, Applicants respectfully request that the rejection of claims 2-3 and 5-10 under 35 U.S.C. §112, first paragraph, be withdrawn.

The 35 U.S.C. §103(a) Rejection

Claims 1-2 was rejected under 35 U.S.C. §103(a) as being unpatentable over **Seth** et al. in view of **Sato** et al. and **Anton** et al. The rejection is moot because claims 1 and 2 have been cancelled.

This is intended to be a complete response to the Final Office Action mailed August 28, 2002. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: Feb 3, 2003

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 3 has been amended as follows:

3. (twice amended) A method of treating an individual having a neoplastic disease, comprising the step of administering to said individual a ~~an amount of the~~ composition comprising an inducible recombinant adenoviral vector encoding a pro-apoptotic *bax* gene which is placed downstream of a loxP excision cassette and a vector encoding a protein that induces the expression of said *bax* gene, wherein the expression of said *bax* gene would induce apoptosis and inhibit tumor cell growth. ~~of claim 2 effective to inhibit neoplastic growth.~~

Claim 7 has been amended as follows:

7. (twice amended) A method of treating an individual having ovarian cancer, comprising the step of administering to said individual a composition comprising an inducible recombinant adenoviral vector encoding a pro-apoptotic *bax* gene which is placed downstream of a loxP excision cassette and a vector encoding a protein that induces the expression of said *bax* gene, wherein the

expression of said *bax* gene would ~~an amount of the composition of claim 2 effective to~~ inhibit ovarian cancer growth.

Claim 9 has been amended as follows:

9. (twice amended) A method of sensitizing tumor cells to chemotherapy ~~and/~~or radiotherapy in an individual, comprising the step of administering to said individual a composition comprising an inducible recombinant adenoviral vector encoding a pro-apoptotic *bax* gene which is placed downstream of a loxP excision cassette and a vector encoding a protein that induces the expression of said *bax* gene, wherein the expression of said *bax* gene would ~~an amount of the composition of claim 2 effective to~~ sensitize said tumor cells.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Curiel *et al.*	§	**ART UNIT: 1632**
	§	
FILED: September 9, 1999	§	
	§	
SERIAL NO.: 09/393,173	§	**EXAMINER:**
	§	**Wehbe, A.M.S.**
FOR: Adenoviral Vector Encoding Pro-Apoptotic Bax Gene and Uses Thereof	§	
	§	
	§	**DOCKET: D6163**

Assistant Commissioner for Patents
BOX AF
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, David T. Curiel, do hereby state as follows:

I am a co-inventor of the above-referenced patent application. I have read U.S. patent application serial no. 09/393,173 and I am aware of the content of the Office Action, including all prior art cited against the '173 application.

An issue relating to the patentability of the claimed methods is the degree of enablement provided by Applicants' specification. The following data are presented as evidence of enablement commensurate with the scope of the claims:

To demonstrate the capacity of adenovirus to deliver *bax* *in situ* and to sensitize previously irradiated tumor, an *in vivo* therapeutic experiments were performed. Nude mice (n=5/group) bearing established subcutaneous human glioblastoma cells D54MG received either radiation alone, irrelevant virus (Ad/Bax+Ad/Luc) with radiation, or Ad/Bax+Ad/Cre with or without radiation. Radiation (5 Gy) and viruses were administered every other day for four times.

Animals treated with Ad/Bax+Ad/Cre without radiation, as well as those non-treated, showed rapid tumor growth, and had to be sacrificed after 5 weeks (Fig. 1). Animals treated with radiation alone or radiation with irrelevant viruses showed a transient inhibition of their growth although the tumor grew back aggressively, and the animals had to be sacrificed after 8-10 weeks. In contrast, animals treated with Ad/Bax+Ad/Cre and radiation showed significant regression and inhibition of tumor growth over a 6-month time period. Of these mice, 60% had no evidence whatsoever of tumor. Thus, combined treatment of *bax* and radiation is uniquely able to completely eradicate malignant glioma tumor nodules in this mice model.

To show that Bax induces apoptosis in normal human astrocytes, and to demonstrate that it has sensitizing effects to radiation, astrocytes were exposed to 0 or 8 Gy radiation and infected with 100 PFU of Ad/Bax+Ad/Cre or irrelevant viruses (Ad/CD+Ad/Cre). Six days later, apoptosis was determined. Although Bax induced some apoptosis over that observed in uninfected controls (24% vs. 10% respectively), the levels were only slightly higher than those induced by irrelevant control viruses (18%) (Fig. 2A). Most importantly, the addition of radiation provoked a minor and insignificant ($p=0.267$) increase in apoptosis. This effect was small compared to the much greater increase of apoptosis observed with radiation in all the glioblastoma cell lines examined. To confirm this result, a cell proliferation assay was performed. Astrocytes treated with Ad/Bax and Ad/Cre or irrelevant virus showed no significant inhibition of cell proliferation (Fig. 2B). In addition, cell proliferation of irradiated cells did not differ significantly with or without viral treatment. Thus, Bax does not appear to sensitize normal astrocytes to the effect of radiation.

In conclusion, these data demonstrate a synergistic radiosensitizing effect of Bax in refractory glioblastoma cell lines after gene delivery

via recombinant adenovirus. This result was confirmed in an *in vivo* murine xenograft model of glioblastoma. Toxicity was selectively induced in tumors, but normal astrocytes were spared. Thus, the combination of *bax* gene delivery and radiotherapy might have clinical utility for the treatment of malignant brain tumors. Accordingly, I respectfully submit that the scope of the claims 2-3 and 5-10 in the '173 application has a reasonable correlation to the scope of the enablement provided.

Figure Legends

Figure 1: Subcutaneous nodules of glioma were radiosensitized by intratumoral delivery of Bax. In this experiment, nude mice 4 to 6 week old were used. Animals were classified in 5 groups (n=5) according to treatment as follows: radiation alone, irrelevant virus with or without radiation (5 Gy x 4), and Ad/Bax+Ad/Cre with or without radiation (5 Gy x 4). Animals were injected subcutaneously into lower flanks with 2 x 10^7 D54MG cells, and then followed for nodule formation for 3 weeks. When nodules reached suitable size, the tumors were irradiated and viruses were injected intratumorally at an MOI of $1x10^9$ following each

administration of radiation. Nodules were then monitored up to 6 months for tumor size. Representative data from one of two similar experiments is shown.

Figure 2:Bax does not sensitize normal human astrocytes to radiation. Human astrocytes (2 x 10^5/well) were plated into 6-well plates, and irradiated with 8 Gy of ^{60}Co irradiation or mock irradiated 5 days later. Apoptosis was determined 6 days after irradiation (Fig. 2A). Results from a cell proliferation assay was shown in Fig. 2B.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or patent issued thereon.

Date: 1/30/03

Dr. David T. Curiel

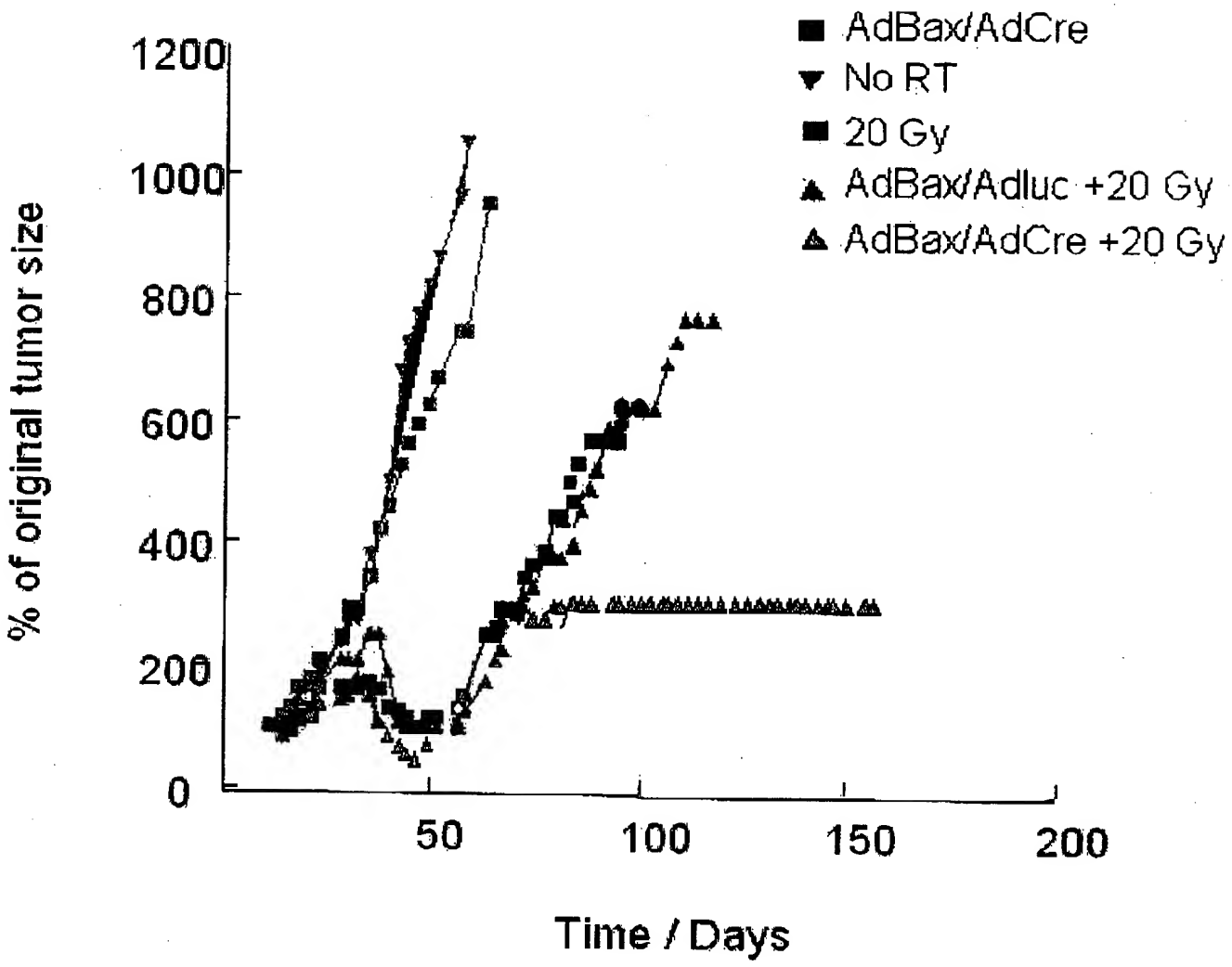
AdBax/AdCre
No RT
20 Gy
AdBax/Adluc +20 Gy
AdBax/AdCre +20 Gy
% of original tumor size
1200
1000
800
600
400
200
0
50
100
150
200
Time / Days

Figure 1

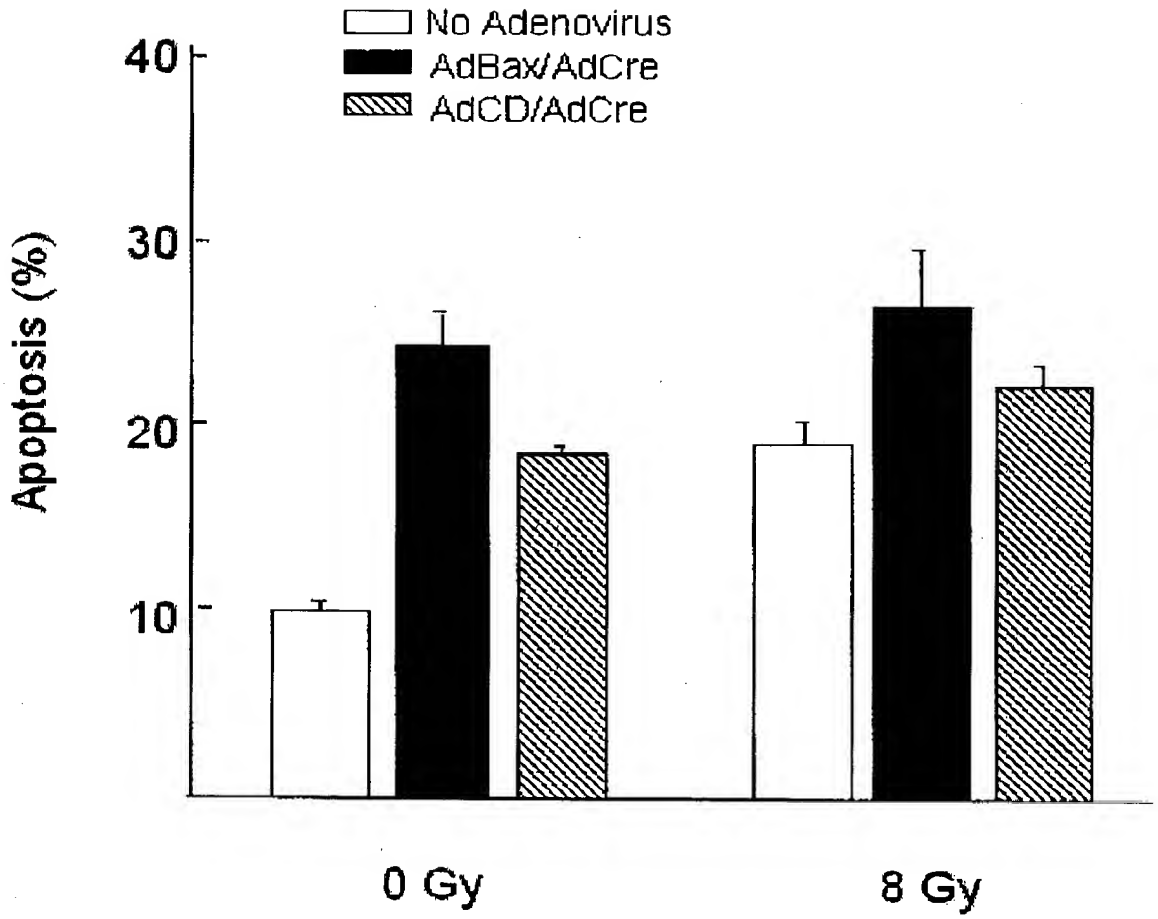
No Adenovirus
AdBax/AdCre
AdCD/AdCre
Apoptosis (%)
40
30
20
10
0 Gy
8 Gy

Figure 2A

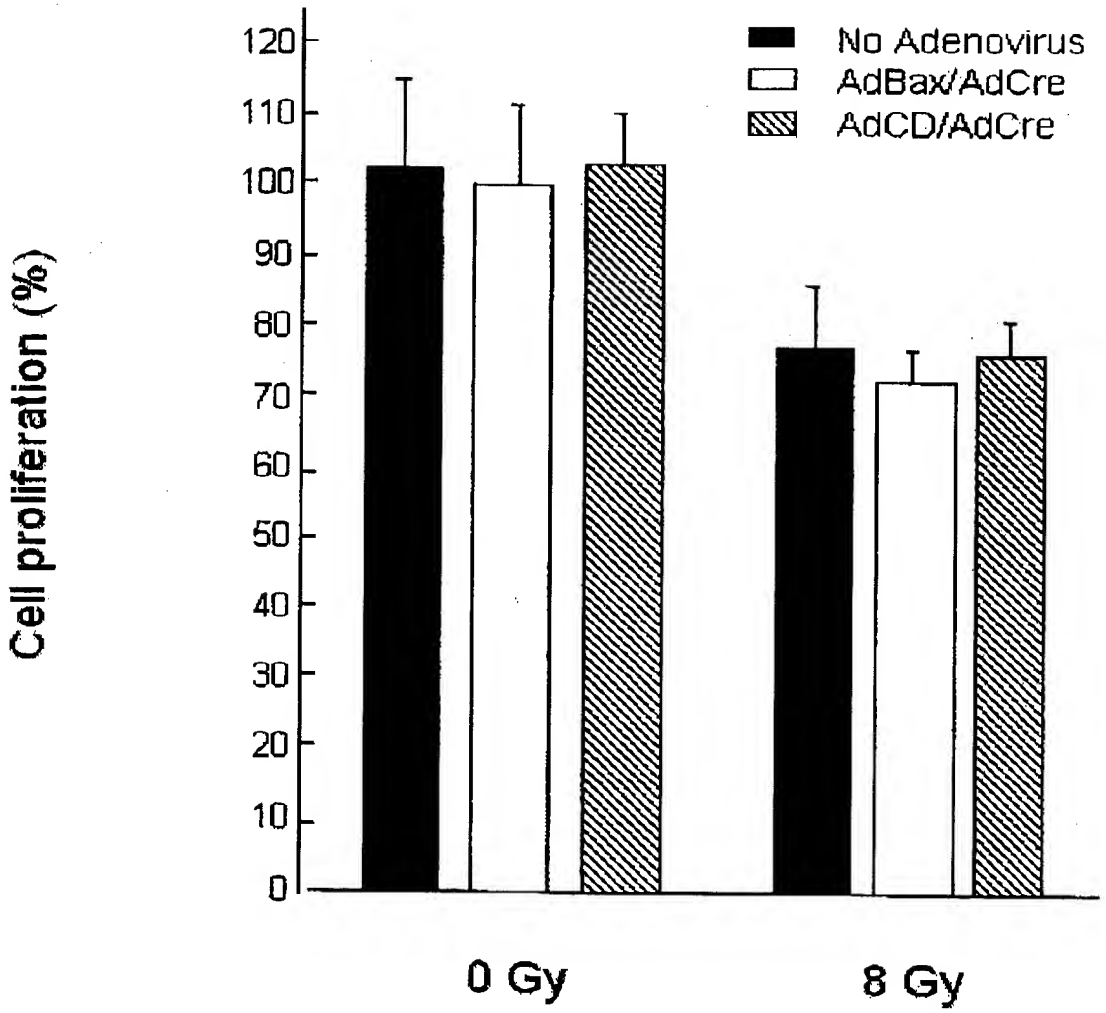
Cell proliferation (%)
120
110
100
90
80
70
60
50
40
30
20
10
0
No Adenovirus
AdBax/AdCre
AdCD/AdCre
0 Gy
8 Gy

Figure 2B